

CLAIMS

What is claimed is:

1. A method for cleaving cellular chromatin in a region of interest, the
5 method comprising:
 - (a) selecting the region of interest;
 - (b) engineering a first zinc finger binding domain to bind to a first nucleotide
sequence in the region of interest;
 - (c) providing a second zinc finger binding domain which binds to a second
10 nucleotide sequence in the region of interest, wherein the second sequence is located
between 2 and 50 nucleotides from the first sequence;
 - (d) expressing a first fusion protein in the cell, the first fusion protein
comprising the first zinc finger binding domain and a first cleavage half-domain; and
 - (e) expressing a second fusion protein in the cell, the second fusion protein
15 comprising the second zinc finger binding domain and a second cleavage half domain;
wherein the first fusion protein binds to the first nucleotide sequence, and the
second fusion protein binds to the second nucleotide sequence, and further wherein
said binding positions the cleavage half-domains such that the cellular chromatin is
cleaved in the region of interest.
- 20 2. The method of claim 1, wherein cleavage occurs between the first and
second nucleotide sequences.
3. The method of claim 1 wherein the second zinc finger binding domain
25 is engineered to bind to the second nucleotide sequence.
4. The method of claim 1 wherein the first and second cleavage half-
domains are from the same endonuclease.
- 30 5. The method of claim 4 wherein the endonuclease is a Type IIS
restriction endonuclease.
6. The method of claim 5 wherein the Type IIS restriction endonuclease
is *Fok I*.

7. The method of claim 1 wherein the cellular chromatin is in a chromosome.

5 8. The method of claim 1, wherein the first cleavage half domain is from a Type IIS restriction endonuclease.

9. The method of claim 1, wherein the second cleavage half domain is from a Type IIS restriction endonuclease.

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10. The method of claim 1, wherein the first and second nucleotide sequences are on opposite strands of DNA.

11. The method of claim 10, wherein, in the first and second fusion
15 proteins, the cleavage half-domains are closer to the N-termini and the zinc finger binding domains are closer to the C-termini.

12. The method of claim 1, wherein the first and second nucleotide sequences are on the same strand of DNA.

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13. The method of claim 12 wherein, in the first fusion protein, the cleavage half-domain is closer to the N-terminus and the zinc finger binding domain is closer to the C-terminus and, in the second fusion protein, the zinc finger binding domain is closer to the N-terminus and the cleavage half-domain is closer to the C-
25 terminus.

14. The method of claim 12 wherein, in the first fusion protein, the zinc finger binding domain is closer to the N-terminus and the cleavage half-domain is closer to the C-terminus and, in the second fusion protein, the cleavage half-domain is
30 closer to the N-terminus and the zinc finger binding domain is closer to the C-terminus.